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Original Research Article

Vitamin B2 enhances development of puberty ovaries via regulation of essential elements and plasma endocrine hormones

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Abstract

Purpose: *To investigate the effect of vitamin B2 (VB2) on ovarian development during puberty.*
Methods: *Four groups of domestic hens (Jinghong-1 strain, 12 hens/group) were housed under standard conditions and fed basal diet with or without graded doses of VB2 (10 – 40 mg/kg). At 10 weeks old, 9 hens were sacrificed from each group. Plasma levels of AST, ALT, steroid hormones and growth hormones were determined. In addition, some essential mineral elements in the ovarian tissue of the hens were assayed.*
Results: *Treatment with VB2 significantly improved ovary and liver organ indices ($p < 0.01$), but had no deleterious effect on the liver. The different doses of VB2 exerted regulatory effects on homeostasis of essential elements in the ovary ($p < 0.01$). Moreover, VB2 treatment elevated plasma levels of progesterone (PR) and estrogen (ES), suggesting that it might regulate steroid hormone levels.*
Conclusion: *These results indicate that VB2 enhances the development of the ovaries during puberty.*

Keywords: *Domestic hen, Ovarian development, Vitamin B2, Steroid hormones, Mineral elements*

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INTRODUCTION

Adolescence is a crucial period in the development of reproductive organs in the female species [1]. Significant developmental changes occur in the reproductive system after birth. Therefore, the European Drug Administration (EMA) and the Food and Drug Administration (FDA) have advised that drugs can pose a highly toxic risk to the reproductive system [2]. The ovaries which produce eggs are an integral part of the female reproductive system [3]. The development of the ovaries during puberty needs many nutrients, growth factors and endocrine hormones [4]. Several hormones and growth factors can regulate ovarian development through the control of levels of interleukins in ovarian tissue (IL-1, IL-6, TNF and IL-2), as well levels of sex hormones and cortisol in the blood [5]. However, there are no in-

depth investigations on the effect of VB2 on changes in the ovaries during development.

Vitamin B2 (VB2, Figure 1), also called riboflavin, can be found in vegetables and animal tissues. It is important in several biochemical processes such as oxidative metabolism, cell growth and vitamin B6 metabolism. It is also required for absorption, storage and mobilization of iron [6]. Some reports have suggested that VB2 possesses antioxidant properties [7].

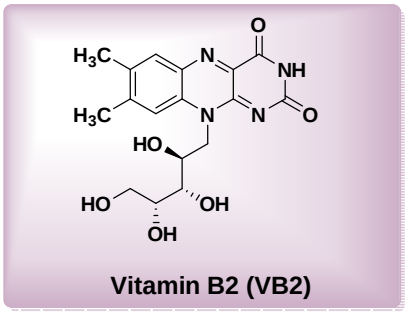

Figure 1: The structure of Vitamin B2

The chicken (*Gallus gallus*) forms a link in the evolution gap between mammals and other vertebrates [8]. Embryos of chicken are very good and attractive model for use by researchers in the field of embryology and developmental biology, particularly their ovaries [9]. Some research has shown that ovaries from mammals are rich in sensory and sympathetic neurons outside the gland, and there is also internal innervation through the gland neurons. So far, there are no reports on the effect of VB2 on essential element levels in the ovary, and on endocrine hormones in the blood. Therefore, the aim of the present was to use the chicken ovary as an endocrine research model to investigate the effects of VB2 on the levels of ovarian essential elements and plasma hormones during puberty.

EXPERIMENTAL

Experimental design and sample collection

The experiments were carried out in Maochangyuan Co., Qiangdao, China, in a commercial poultry house. The domestic hens were housed in a conventional and enclosed henhouse with lighting programmed to 16 h:8 h light : dark cycles, in an atmosphere with a relative humidity of 55 %. The hens were permitted *ad libitum* access to a basal feed composed from soybean and corn [10], and clean drinking water. They were randomly assigned to four groups (12/group): control group (fed basal diet only), and three other groups fed basal diet plus VB2 at 10, 20 or 40 mg/kg. There were three replicates per group, and the hens were weighed at the start and end of the experiment in order to determine body weight changes. Weekly feed consumption was calculated. After 10 weeks of treatment, 9 hens were sacrificed from each group. Blood was collected in anticoagulated bottles for plasma samples, and ovarian tissue samples were excised. The tissue and plasma samples were deep-frozen at -80 ^{o}C prior to assays.

This study received approval from the Ethical Committee of School of Life Science, Jilin University, Qianjin Street 2699, ChangChun, Jilin, 130012, China (approval no. 20185302), and it was carried out in accordance with the guidelines of Helsinki Declaration of 1964, as amended in 1996 [10].

Determination of mineral contents of ovarian tissue

Weighed samples of ovarian tissue were subjected to acid digestion and the digests were used for the assay of iron (Fe), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), zinc (Zn), phosphorus (P) and copper (Cu) using inductively coupled plasma atomic emission spectroscopic (ICP-OES) procedure.

Determination of plasma ALT and AST levels

Plasma ALT and AST were assayed colorimetrically with kits (Nanjing, China).

Measurement of plasma steroid hormones and growth hormone

ELISA kits (Nanjing, China) were used for assaying estrogen (ES) and progesterone (PR) in plasma.

Statistical analysis

The data were analyzed statistically using GraphPad Prism 6.0 software. Two-group comparisons were done with one-way ANOVA and Tukey's multiple comparison. Values of $p < 0.05$ were deemed significant.

RESULTS

Effect of different VB2 treatments on ovary or liver organ index

As shown in Figure 2, ovary index was significantly decreased by exposure to 10 mg/kg VB2 ($p < 0.05$), but was significantly increased by administration of 40 mg/kg VB2 ($p < 0.01$). No

appreciable effect was produced by VB2 at a dose of 20 mg/kg. However, liver index was significantly raised dose-dependably by VB2 at doses of 20 and 40 mg/kg, relative to the untreated group.

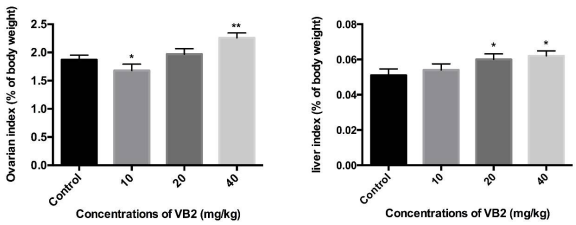

Figure 2: Effect of different doses of VB2 on ovary and liver indexes. Values are presented as mean ± SD (n = 9); *p < 0.05, **p < 0.01, compared to control

Effect of VB2 treatments on liver

The activity of AST was significantly decreased by treatment with VB2 at doses of 20 and 40 mg/kg. However, only VB2 produced no effect on ALT at doses of 10 and 40 mg/kg, while a significant increase was produced by exposure to 20 mg/kg, relative to untreated control group (Figure 3).

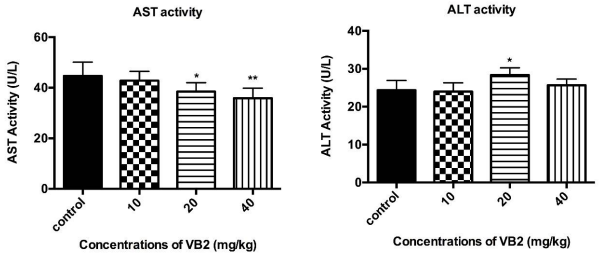

Figure 3: Effect of VB2 treatment at different doses ALT and AST. Values are expressed as mean ± SD (n = 9); *p < 0.05, **p < 0.01, compared to the control group

Influence of VB2 on ovarian levels of some essential elements

The ovarian levels of Ca, Mn, K, Fe, Mg, Cu and Zn increased significantly at VB2 doses of 10 and 20 mg/kg, but dipped at 40 mg/kg, when compared with the untreated control group (p < 0.05). The pattern of changes in all the levels of the mineral elements were similar, except for P, which increased in concentration with increase in VB2 dose. These results, which are shown in Figure 4, indicate that VB2 exerts a regulatory influence on homeostasis of elements in the ovarian tissue.

Effect of VB2 treatments on plasma steroid hormone levels

As indicated in Figure 5, the low-dose VB2 treatment caused no appreciable changes in plasma levels of ES and PR. However, VB2 at medium and high doses, led to significant increases in the plasma levels of these hormones, when compared with the control group.

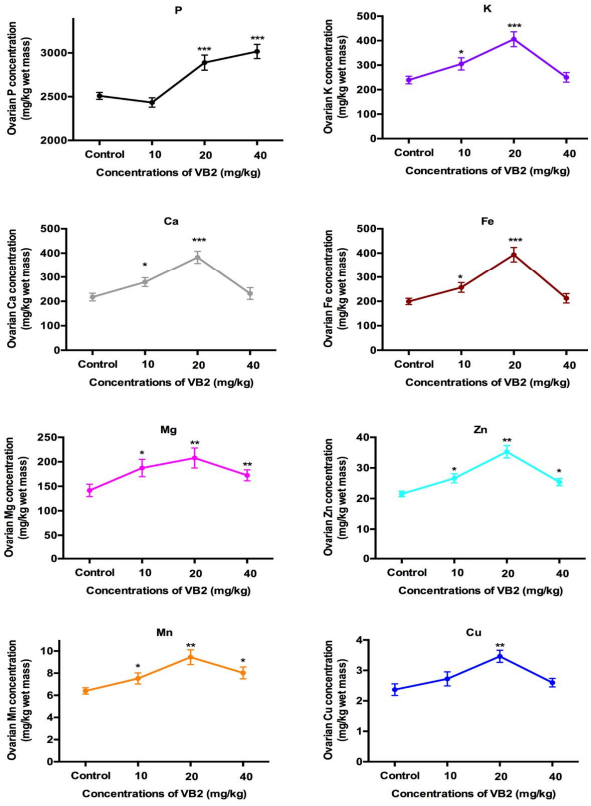

Figure 4: Effect of VB2 treatments on essential elements in the ovary. Each point is mean ± SD (n = 9); *p < 0.05, **p < 0.01 and ***p < 0.001, compared with control

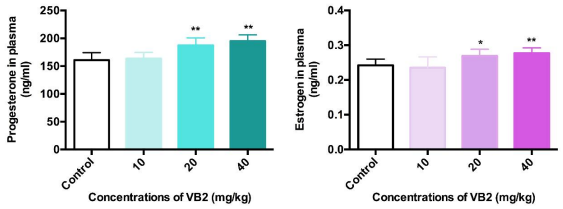

Figure 5: Effect of VB2 treatments on plasma levels of progesterone and oestrogen. Data are expressed as mean ± SD (n = 9); *p < 0.05, compared to control

DISCUSSION

The effects of VB2 on ovarian essential elements and plasma steroid hormone levels have received very little research attention. In the

present study, pubertal hens were exposed to different levels of VB2. At a dose of 40 mg/kg, VB2 treatment resulted in slightly increased ovarian and liver organ indexes which were significantly higher than the corresponding indexes in the control group. Interestingly, low dose of VB2 (10 mg/kg) resulted in reduction of ovarian index.

Liver cells contain ALT and AST [11]. During liver damage, these transaminases leak out into the blood, resulting in elevations of blood ALT and AST, which are used as markers of liver injury. Alanine transaminase (ALT) is the more sensitive of the two, and its activity in liver tissue is 100 times greater than that in the serum [12]. Thus, in most cases, the levels of ALT and AST are consistent with the degree of hepatocyte damage [13].

Compared to the control, 10 mg/kg VB2 treatment had no effect on AST and ALT activities. Besides, increased doses of VB2 resulted in decreases in plasma AST activity, while 20 mg/kg VB2 treatment significantly elevated ALT activity, although the activity was within the safe range. Thus, the two vital indicators of liver damage were not adversely affected, suggesting that high-dose VB2 treatment might be relatively safe.

Essential elements are required for human growth and development, and for maintaining normal physiological functions. They affect the normal functioning of the endocrine glands, the growth of organs, and the biological effects of hormones [14]. In view of these functions, the essential elements P, K, Ca, Fe, Mg, Zn, Mn and Cu were determined in the ovary samples. Relative to the control group, VB2 increased concentrations of K, Ca, Fe, Mg, Zn, Mn and Cu in the ovary, with peak values at the dose of 20 mg/kg., followed by a decrease at the dose of 40 mg/kg. However, P levels increased at higher doses VB2 treatment.

The elemental analyses were carried out to determine the effect of VB2 on the ovarian levels of these elements, and the correlation between the elemental concentration and VB2 dose. The development of the ovaries is an extremely complex process [15]. Essential elements, steroid hormones, and neuronal factors combine in promoting ovarian development and growth [16]. The two steroidal hormones, ES and PR [17,18], which are essential for pubertal ovarian development, were not affected by 10 mg/kg VB2 treatment. However, the 20 and 40 mg/kg VB2 treatment elevated the plasma concentrations of ES level and PR. This elevation was aimed at releasing more hormones for ovarian growth. The data from this study suggest that VB2 promotes ovarian development by regulating ovarian neuronal factors and plasma steroid hormone levels.

CONCLUSION

Vitamin B2 (VB2) exerts an effect on development of the ovaries of domestic hens during puberty. It regulates ovarian neuronal factors and plasma steroid hormone levels, which include the levels of essential elements, progesterone and oestrogen. Moreover, VB2 is safe for the liver, even at high doses. Thus, these results suggest that VB2 may promote the development of ovaries during puberty.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

This work was done by the authors named in this article and the authors accept all liabilities resulting from claims relating to this article and its contents. The study was conceived and designed by Xiaobo Liu; MuXin Li, Haifeng Zhao, Junhan Hu, Le Zhou, YongMei Sun, Xiaobo Liu analysed the data; MuXin Li wrote the manuscript. All authors read and approved the publication of the manuscript.

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